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Notes on rust fungi in China 9. *Puccinia miscanthi* life cycle and morphology confirmed by inoculation

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Abstract—The Heteroecious and macrocyclic life cycle of *Puccinia miscanthi* is confirmed for the first time with inoculation experiments in China. The rust produces spermogonial and aecial stages on *Plantago asiatica* and uredinial and telial stages on *Miscanthus sacchariflorus*. Morphological characters of these stages are described based on field collections and specimens that confirmed their life cycle connections. The neotype of *P. miscanthi* is designated.

Key words—*Plantaginaceae*, plant disease, *Poaceae, Pucciniales, Uredinales*

Introduction

Puccinia miscanthi was first described with uredinial and telial stages on *Miscanthus sacchariflorus* (*Poaceae*) collected in northeastern China (Miura 1928). The life cycle of *P. miscanthi* was reported in Japan by Hiratsuka (1933), Ito (1934), and Asuyama (1936) under the name of *P. eulaliae* Barclay. These authors demonstrated life cycle connection between spermogonial and aecial stages on *Plantago asiatica* (*Plantaginaceae*) and uredinial and telial stages on *M. sinensis* Andersson through inoculations. Ito (1950) considered the rust fungus as producing spermogonial and aecial stages on *Plantago* and uredinial and telial stages on *Miscanthus* and *Imperata* (as *P. eulaliae*) and treated *P. miscanthi* as a synonym of this species. Later, however, this rust fungus

was considered to represent a species different from *P. eulaliae* because of morphology and host relations, and it was treated as *P. miscanthi* (Cummins 1971, Hiratsuka & al. 1992). Sato & Kakishima (1982) reported *Lysimachia clethroides* Duby (*Primulaceae*) as an additional host of the rust's spermogonial and aecial stages. This rust fungus has been reported on wide range of host plants from northeastern Asia (Table 1, p. 532).

During investigation of rust fungi in northeastern China, a *Puccinia* species producing uredinial and telial stages on *M. sacchariflorus* was collected in Heilongjiang Province. Spermogonial and aecial stages of a rust fungus on *Plantago asiatica* were also found near infected *M. sacchariflorus* plants. The spermogonial and aecial stages were suspected to represent different stages of *Puccinia miscanthi.* However, life cycle connection of *P. miscanthi* between different host plants has not been clarified in China, although *Pl. asiatica, Pl. depressa* Willd., and *Pl. major* L. have all been listed as spermogonial and aecial host plants, and *M. floridulus* (Labill.) K. Schum. & Lauterb., *M. sacchariflorus, M. sinensis, Saccharum spontaneum* L., and *Thysanolaena maxima* (Roxb.) Kuntze as uredinial and telial hosts (Wang & Zhuang 1998, Cao & Li 1999). Furthermore, *Puccinia cynodontis* Lacroix ex Desm. also has been reported to produce spermogonia and aecia on *Plantago* species including *Pl. asiatica* (Cummins 1971, Wang & Zhuang 1998). Worldwide, uredinial and telial stages of six morphologically similar *Puccinia* species have been recorded on *Miscanthus* (Table 1; Cummins 1971, Hiratsuka & al. 1992, Wang & Zhuang 1998). Among them, *P. miscanthi, P. miscanthicola* F.L. Tai & C.C. Cheo, *P. melanocephala* Syd. & P. Syd., and *P. erythropus* Dietel have been recorded in China (Tai 1979, Wang & Zhuang 1998). Therefore, we carried out inoculation experiments to clarify the life cycle connection between spermogonia and aecia on *Pl. asiatica* and uredinia and telia on *M. sacchariflorus* collected in Heilongjiang Province on 2 July 2018. We also observed morphology of these specimens for identification of species.

Materials & methods

Inoculation experiments

On 2 July 2018 uredinia and telia on *Miscanthus sacchariflorus* were found at Wuchang, Heilongjiang Province, China (44°12′22″N 128°03′33″E). Infected plants were transplanted in plastic pots and placed on the ground at the campus of Jilin Agricultural University, Changchun, Jilin Province. In late summer, abundant telia appeared on the plants, and these telia were overwintered on dead leaves. Teliospores obtained from the telia were observed to germinate and basidiospores produced in the spring of 2019 were used for inoculations. On 17 June 2019 small pieces of leaves

Fig. 1. *Puccinia miscanthi* on *Plantago asiatica* (A, C, E) and *Miscanthus sacchariflorus* (B, D, F). A. The plants in plastic pots, producing spermogonia and aecia by basidiospore inoculation. B. Plant transplanted from Heilongjiang Province and cultured at campus of Jilin Agricultural University. C. Spermogonia produced on leaf surface by basidiospore inoculation. D. Brown uredinia produced on leaf surface by aeciospore inoculation. E. Aecia on lower leaf surface produced by basidiospore inoculation. F. Dark brown telia on leaf.

with teliospores were attached to the surfaces of young healthy leaves of *Pl. asiatica* for inoculation, using a similar method previously reported by Ji & al. (2017a,b). The inoculated plants were collected at campus of Jilin Agricultural University and had been maintained in plastic pots. No natural infection of the field plants had

been confirmed prior to inoculation. The inoculated plants were kept in a humid environment in a plastic box in darkness at 20–23 °C for 3 days and then transferred to a place near windows at 20–23 °C for observation.

Aeciospores obtained from aecia on the basidiospore-inoculated *Plantago asiatica* leaves were dusted onto small pieces of wet filter paper (about 3 mm^2) in a Petri dish. These papers were then placed on young healthy leaves of *M. sacchariflorus,* which had been transplanted from Wuchang, Heilongjiang Province in 2018 and maintained in plastic pots at campus of Jilin Agricultural University. Plants with new leaves were used for the inoculation. The inoculated plants were kept in a humid environment in a plastic box under the same conditions as the basidiospore inoculations, and later transferred to the place near windows.

Morphological observations

Specimens collected at Wuchang, and specimens obtained by inoculations were morphologically examined. The spores and size and shape of sori were microscopically examined using Light (LM) and scanning electron (SEM) microscopes according to Ji & al. (2019). The specimens are deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, China (HMJAU).

Results & discussion

Life cycle

About 10 days after inoculation of *Plantago asiatica* with basidiospores from germinating teliospores, small yellow spots of spermogonia appeared on both surfaces of the inoculated leaves (Figs 1A, C). These spots were frequently observed on the leaf, and the leaf tissue around infected veins was sometimes malformed when many spots were produced. About 10 to 12 days later, orange-yellow aecia were produced around the spots on lower surfaces of the leaves (Fig. 1E).

About 5 days after aeciospore inoculation, pale yellow spots appeared on the leaves of *Miscanthus. sacchariflorus*, after which then brown uredinia were produced (Fig. 1D). One to two months after that, blackish telia appeared on *M. sacchariflorus* (Fig. 1F). The inoculation results confirmed that the rust fungus producing spermogonial and aecial stages on *Pl. asiatica* was conspecific with the rust fungus producing uredinial and telial stages on *M. sacchariflorus.*

Morphology & identification

The inoculations show that this rust fungus has an heteromacrocyclic life cycle, producing spermogonia and aecia on *Plantago asiatica* and uredinia and telia on *Micanthus sacchariflorus.* The uredinial and telial stages of our Heilongjiang Province rust on *M. sacchariflorus* are morphologically similar to those of

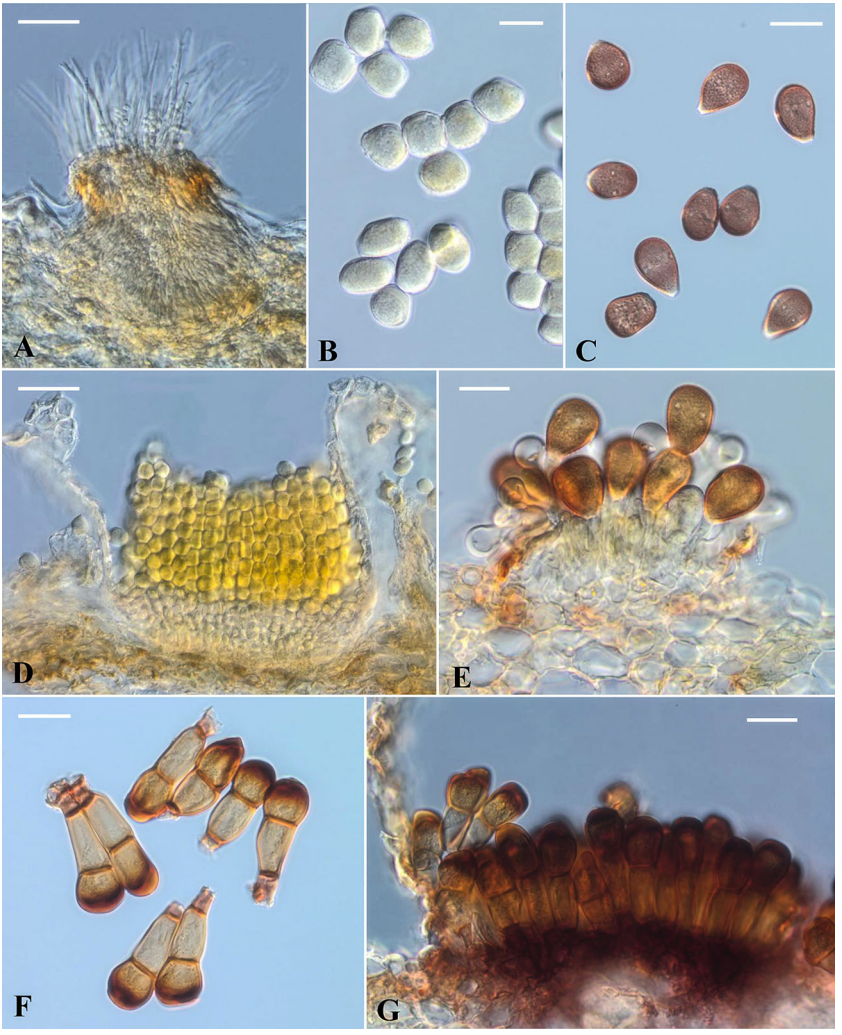

Fig. 2. *Puccinia miscanthi* on *Plantago asiatica* (HMJAU8642: A, B, D) and *Miscanthus sacchariflorus* (neotype, HMJAU8643: C, E, F, G) observed by LM. A. Vertical section of spermogonium (type 4). B. Aeciospores with verrucose surface. C. Urediniospores with equatorial germ pores. D. Vertical section of aecium with catenulate aeciospores surrounded by peridium. E. Vertical section of uredinium mixed with paraphyses. F. Teliospores. G. Vertical section of telia, surrounded by host epidermis. Scale bars: A, C = 30 μm; B, E = 20 μm; D = 100 μm; F, G = 25 μm.

Puccinia erythropus, *P. melanocephala*, and *P. miscanthi* in urediniospore and teliospore shapes. However, teliospores of *P. erythropus* and *P. melanocephala*

are shorter, and their apical walls thinner, than those of our *M. sacchariflorus* rust fungus (Table 1). Furthermore, although Ito (1950) described paraphyses (Table 1), *P. erythropus* has been reported to have no paraphysis in uredinia, whereas, our *M. sacchariflorus* rust fungus produces abundant paraphyses (Figs 2E, 3D). The morphological characters of our *M. sacchariflorus* rust fungus are very close to those previously reported for *P. miscanthi*, but as its teliospores are only slightly shorter than these descriptions (Table 1), we identified our rust fungus as *P. miscanthi.* Host range and morphological features of *P. miscanthi* reported by different authors as shown in Table 1; the differences may be considered variations within species caused by host plants and geographical distribution. *Puccinia miscanthi* has been reported to be genetically very close to *P. melanocephala* although they are morphologically different (Virtudazo & al. 2001, Dixon & al. 2010). Phylogenetic analyses with specimens from different regions and host plants may clarify their relationships.

Spermogonial and aecial stages of *Puccinia miscanthi* have been reported to be produced on *Plantago* and *Lysimachia* species (Cummins 1971, Hiratsuka & al. 1992, Wang & Zhuang 1998). However, no confirmation of these stages of *P. miscanthi* has been reported in China, although its life cycle connection was reported in Japan with inoculations. Therefore, *Pl. asiatica* is the first host plant confirmed by inoculations of its spermogonial and aecial stages in China. The morphology of these stages is almost identical with the descriptions reported from Japan by Ito (1950), Sato & Kakishima (1982), and Hiratsuka & al. (1992), except for the slightly smaller aeciospores. Wang & Zhuang (1998) described the rust's spermogonial and aecial stages on *Pl. depressa* in China; however, a connection to its uredinial and telial stages was not confirmed. Two *Plantago* species, *Pl. asiatica* and *Pl. lanceolata* L., were also reported as spermogonial and aecial host plants of *P. cynodontis* without confirmation of their life cycle connections in China (Wang & Zhuang 1998). Therefore, rust species identifications of spermogonial and aecial stages on species of *Plantago* should be reconsidered. Ono & Azbukina (1997) reported that *P. erythropus*, morphologically similar to *P. miscanthi*, produced spermogonia and aecia on *Cynanchum sublanceolatum* var. *obtusum* (Franch. & Sav.) Matsum. (*Apocynaceae*), suggesting that this species differs from *P. miscanthi* in its spermogonial and aecial hosts.

Miura (1928) described *Puccinia miscanthi* based on a specimen on *Miscanthus sacchariflorus* collected in Teikaton, Manchuria (northeast China) on 22 September in 1919. However, this specimen has not been found in any herbarium, we therefore designate a neotype. We collected specimens on the

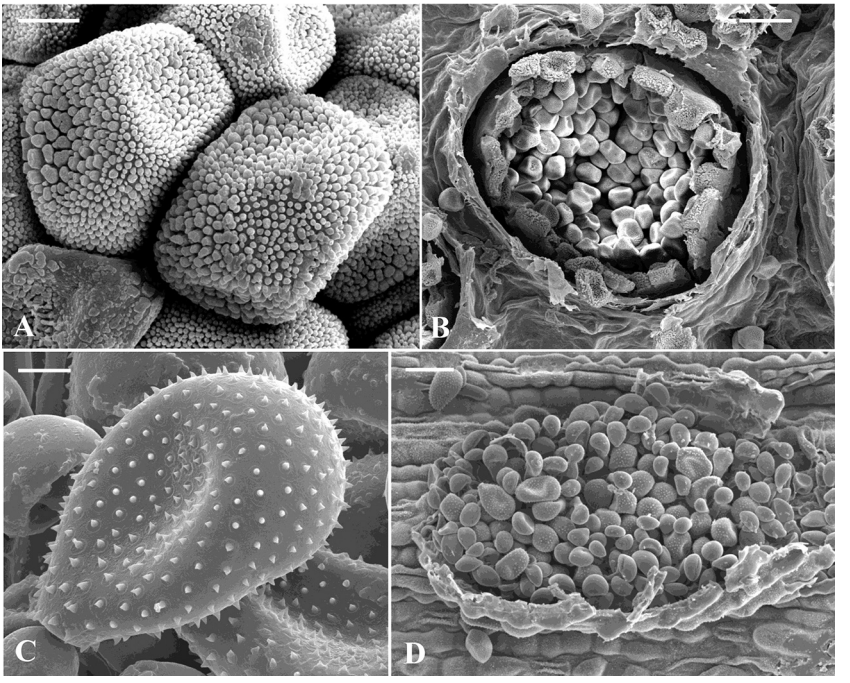

Fig. 3. *Puccinia miscanthi* on *Plantago asiatica* (HMJAU8642: A, B) and *Miscanthus sacchariflorus* (neotype, HMJAU8643: C, D) observed by SEM. A. Aeciospores with densely verrucose surface. B. Aecium with peridium. C. Urediniospore with echinulate surface. D. Uredinium mixed with paraphyses. Scale bars: A, C = 5 μm; B = 40 μm; D = 30 μm.

same host plant from the same area of China, as reported by Miura (1928) and used for inoculations. We believe that specimens collected and obtained from inoculations are morphologically close to the original specimen and describe its morphology based on these specimens.

Puccinia miscanthi Miura,

Fl. Manch. E. Mong., III Cryptogams, Fungi: 302, 1928. Figs 1–3

Type: Uredinia and telia on *Miscanthus sacchariflorus* (Maxim.) Franch. (*Poaceae*), China, Helongjiang Province, Wuchang, 9 September 2017, leg. M. Kakishima & J.X. Ji (**neotype designated here,** HMJAU8643; MBT388544).

Spermogonia amphigenous, surrounded by greenish yellow lesions, densely grouped, yellow, subepidermal, flask-shaped, type 4 of Cummins & Hiratsuka (2003). Aecia hypophyllous, densely grouped, yellow, cupulate with peridia, *Aecidium*-type. Aeciospores catenulate, globose to subglobose,

Table 1. Comparative morphology of uredinia and telia of *Puccinia* species reported on *Miscanthus* spp.

Species	Host genera[1]	Distribution	Paraphyses in uredinium[2]	Urediniospores		Teliospores			Reference[3]
				Size (µm)	Pore no.	Size (µm)	Wall thickness (µm)		
							Side	Apex	
P. miscanthi [= *P. eulaliae* S. Ito, non Barclay]	M	China, Taiwan, Japan, Philippines, Russia	+	27–35.5 × 19.5–23.5	4	39.5–56.5 × 14–22	0.5–1	2.6–6	The present paper
	M		?	33 × 25	10	43–61 × 21–25	?	7–8.5	Miura 1928*
	I, M		+	25–38 × 20–31	4–5	32–76 × 16–24	?	2–7	Ito 1950
	I, M, S		+	29–35 × 19–26	4–5	40–60 × 16–23	1.5–2	4–6	Cummins 1971
	I, M, P		+	29–35 × 19–26	4–5	40–60 × 16–23	1.5–2	4–6	Hiratsuka & al. 1992
	T, M, S		+	25–38 × 20–27	4–5	32–76 × 16–24	1.5–2	2–8	Wang & Zhuang 1998
	M		+	27–38 × 20–32	4–5	32–60 × 16–24	1.5–2	4–6	Azbukina 2005
P. miscanthicola	M	China	Uredinia unknown			32–55 × 15–24	2–3	2–3	Cummins 1971
	M					32–55 × 15–24	2–3	2–4	Wang & Zhuang 1998
P. miscanthidii	M	South Africa	–	24–30 × 19–23	3–4	33–46 × 20–27	2–3	5–9	Cummins 1971
P. melanocephala	?	Worldwide	+	22–40 × 18–25	?	35–45 × 14–20	?	4–6	Sydow & Sydow 1907*
	E, S		+	28–33 × 18–23	4–5	30–43 × 17–21	1.5–2	3–4	Cummins 1971
	S		+	28–33 × 18–23	4–5	30–43 × 17–21	1.5–2	3–4	Hiratsuka & al. 1992
	E, S, M		+	27–34 × 18–24	4–5	30–43× 17–22	1.5–2	3–4	Wang & Zhuang 1998
P. daisenensis	M	Japan, Russia	+	28–34 × 21–28	3–5	42–60 × 15–21	?	9–13.5	Ito 1950
	M		+	26–33 × 19–23	4–5	35–56 × 15–22	1.5–2	7–13	Cummins 1971
	M		+	26–33 × 19–23	4	35–56 × 15–22	1.5–2	7–13	Hiratsuka & al. 1992
P. erythropus	M	China, Taiwan, Japan, Philippines, Russia	?	23–28 × 18–23	4	28–45 × 16–21	?	4	Dietel 1905*
	M		+	28–34 × 20–25	4	32–52 × 14–22	2–3		Ito 1950
	E, M		–	24–33 × 18–23	3–4	33–45 × 16–20	1.5–2.5	3–5	Cummins 1971
	M		–	24–33 × 18–23	3–4	33–45 × 16–20	1.5–2.5	3–5	Hiratsuka & al. 1992
	S, M		–	24–32 × 18–23	3–4	32–45 × 17–21	1.5–2.5	3–5	Wang & Zhuang 1998

1) E: *Erianthus*; I: *Imperata*; M: *Miscanthus*; S: *Saccharum*; T: *Thysanolaena*; P: *Phacelurus*. 2) +: Presence; –: Absence; ?: Not given. 3) *: Original description

angular, 17.5–23.5 × 14.5–19.0 μm (av. = 20.5 × 17. 0 μm); walls hyaline, 0.6–1.2 μm (av.= 0.9 μm) thick, densely verrucose.

Uredinia mostly on abaxial surface, scattered or gregarious, yellow-brown, subepidermal, erumpent, with abundant paraphyses. Paraphyses capitate; walls hyaline, thicker at the apical parts. Urediniospores pedicellate, obovoid to ellipsoid, 27.0–35.5 × 19.5–23.5 μm (av. = 30.0 × 21.5 μm); walls brown, echinulate, 0.7–1.5 μm (av. = 1.0 μm) thick; germ pores mostly 4, equatorial. Telia mostly on abaxial surface, blackish brown, erumpent, pulvinate. Teliospores pedicellate, oblong-clavate, 39.5–56.5 × 14.0–22.0 μm (av. = 47.0 × 19.0 μm); walls chestnut-brown, 0.5–1.5 μm (av. = 1.0 μm) thick at sides, 2.5–6.0 μm (av. 4.0 μm) thick at apices, smooth; pedicels persistent, brown, thick-walled, short.

Additional specimens examined — **CHINA, Heilongjiang Province**, **Wuhan**: Spermogonia and aecia on *Plantago asiatica*, 2 July 2018, (HMJAU8644); cultured at Jilin Agricultural University, Changchun, 5 July 2019 (HMJAU8642). Uredinia and telia on *Miscanthus sacchariflorus*, 2 July 2018 (HMJAU8645); cultured at Jilin Agricultural University, Changchun, 5 July 2019 (HMJAU8646).

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